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Yeasts Associated with Living Plants in the Sandy Coastal Area of the Sea of Japan in North-eastern Provinces of Japan

Masaya HAYASHIBE, Nobundo SANDO, Shōzō AWANO,
Junji KURASAWA and Shinki TAKAHASHI

Laboratory of Applied Microbiology, Faculty of Agriculture, Yamagata University, Tsuruoka Yamagata-ken, 997.

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Along the coastal district of the Sea of Japan in North-eastern provinces of Japan, there are sand dunes extending over wide area. The climate in this district is very different from that of inland districts and the Pacific coast. In this sand dune area, many plants, such as autumn goumi (*Elaeagnus umbellata* Thunb.) and other grasses are planted for erosion control. The present experiments were undertaken to clarify the number and kind of yeasts associated with living plants, and seasonal variations in the number of microorganisms under this characteristic environment. Seasonal variations in the number of microorganisms on the flowers, berries and leaves of goumi were surveyed over two years period, 1968 and 1969, from April till October. The seasonal variations in molds and bacteria showed nearly definite patterns, but a definite pattern as to the number of yeasts could not be obtained from these materials not only in yearly suveys but in many sampling points. This means that the growth of yeasts on these materials is governed by complicated factors. The number of microorganisms from other plants and soil was also examined.

A great number of plant colonizing yeasts were purely isolated from these materials and identified. *Candida*, *Cryptococcus*, *Rhodotorula*, *Torulopsis* and *Sporobolomyces* were found to colonize dominantly on flowers and berries. *Candida*, *Cryptococcus*, *Rhodotorula* and *Sporobolomyces* were the main leaf colonizers. These results agreed with those of other workers in many countries having different climate. It was concluded that the yeast flora under the environment in question is not characteristic one compared with that under other environmental conditions.

Sporobolomyces is one of the representative leaf colonizers. There are few records of their presence in flowers and fruits, whereas the present experiments manifested their presence in flowers. The presence of *Schizosaccharomyces japonicus* which is known as a nutritionally exacting yeast was also revealed.

The climate is hot (above 30°C) and moist (above 70%) in summer in the coastal area of the Sea of Japan in North-eastern provinces of Japan which are situated around lat. 40°N. In winter, on the contrary, much snow falls with violent wind. This coast is occupied by sand dunes, and is planted for erosion control with many plants, such as autumn goumi (*Elaeagnus umbellata* Thunb.), a kind of silverberry, pine trees and many other grasses. The soil in this area is in relatively dried state in spite of high humidity of the atmosphere.

The kind of yeasts colonizing in phyllospheres and their seasonal variation were investigated by many workers (*1*). The authors are interested in flora of yeasts colonizing in phyllosphere in sand dune coast in North-eastern provinces of Japan, because 1) the climatic and other conditions in this area are supposed to be very different from those of inland district, and 2) the sand dunes are characteristic in the coastal district of the Sea of Japan and the plants in the sandy coast are also very characteristic.

The present paper describes the seasonal variation in the number of micro-organisms and the kind of yeasts found in plant materials in the sand dune district.

Methods

Sampling places. Sampling places were mainly the sandy coast of Nanakubo about 18 km north-west of Tsuruoka-shi, near which the branch farm of the university is situated (Fig. 1). A spectacular sight of the sand dune of this district

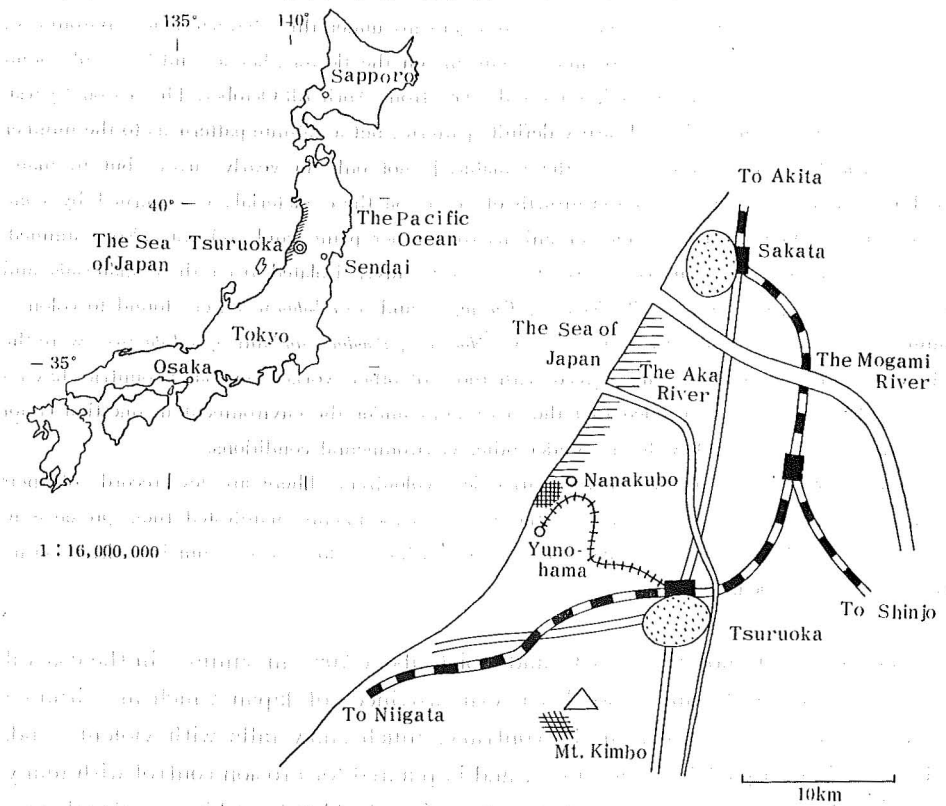

Fig. 1. Situation of the sampling place in North-eastern provinces of Japan.
: Coastal area with sand dunes. : Sampling places.

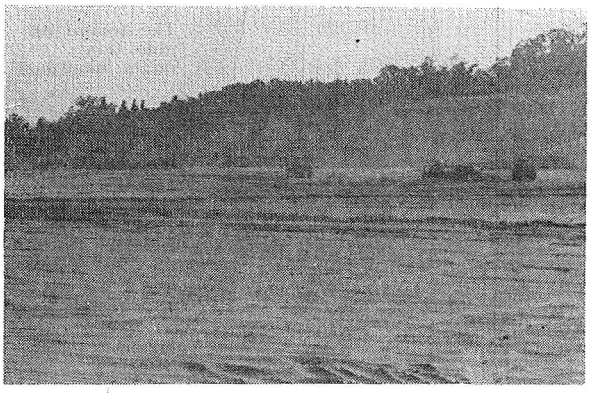

Fig. 2. A spectacular sight of sand dune in the coastal area of the Sea of Japan in North-eastern provinces of Japan. A sand hill near Nanakubo is levelling for sand pit.

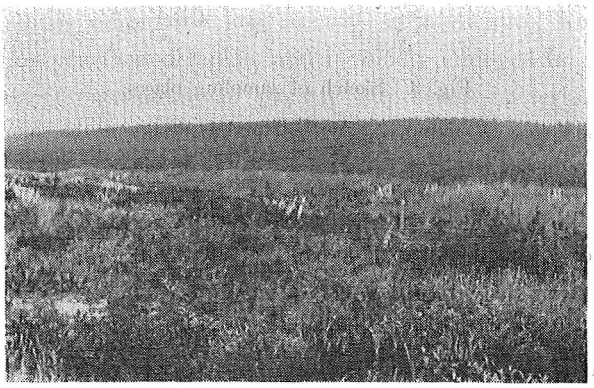

Fig. 4. A view of the sampling place. Photograph was taken in the direction indicated by arrow in Fig. 3.

Fig. 5. A colony of autumn goumi.

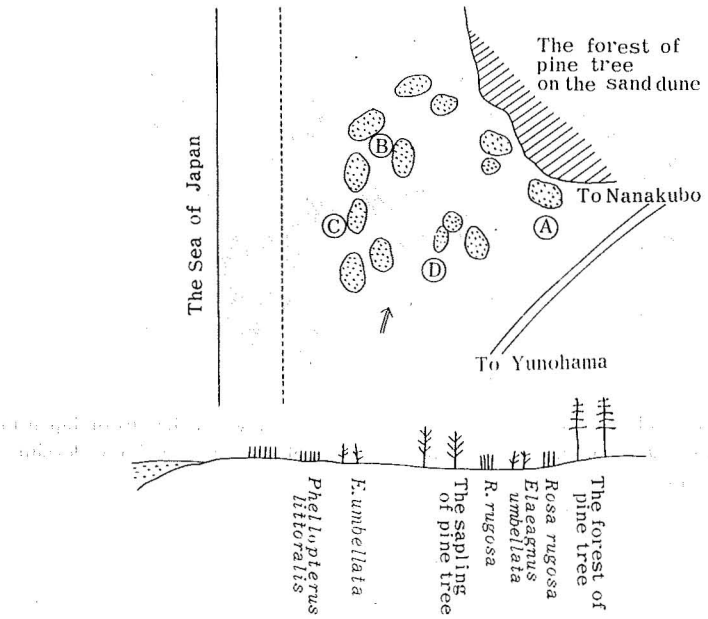

Fig. 3. Sketch of sampling places.

: The colony of goumi. A, B, C and D designate the sampling points.

is shown in Fig. 2. Outline of the sampling places is shown in Figs. 3 and 4. From the front of sandy coast, 2 lines of goumi (Fig. 5) are planted and backward thereof afforestation is made with pine trees, between which many grasses and trees such as Hamabofu (*Phellopterus littoralis* Fr. Schm.), Hamanasu (*Rosa rugosa* Thunb.) *etc.*, and saplings of pine trees are planted. The flowers, leaves and berries of such trees and grasses were sampled from the places designated as A, B, C, and D in Fig. 3.

Sampling materials and sampling method. Thirty pieces of flowers, berries and leaves of the plants, and 1 g of soil under the goumi tree were collected in sterilized test tubes.

Ten milliliters of sterilized water was put into the test tubes containing samples, and shaken well to wash out attached microorganisms. The supernatant liquid was diluted serially with sterilized water and plated out.

Colony counting. Koji extract (Bllg 10°) with 2 % agar (pH 5.0) was mainly employed. Occasionally, koji agar containing 30 μg/ml of dihydrostreptomycin, soil extract agar containing 0.2 g K_2HPO_4 and 15 g agar in 1000 ml of soil extract (pH 6.8) and albumin agar consisted of glucose 1 g, K_2HPO_4 0.5 g, Na_2SO_4 0.2 g, albumin 0.25 g, agar 15 g in 1000 ml of the medium (pH 6.8) were also employed. Soil extract was prepared by extracting 500 g of fertilized soil with 1500 ml of water at 120℃ for 30 min.

One milliliter of the diluted specimens was placed in a petri dish and koji agar medium was poured on it with well rotation in order to disperse the organisms. The petri dishes were incubated at 30°C for 3～5 days and appeared colonies were counted.

Isolation of yeasts. Isolation of yeasts was made by picking up from isolated colonies after microscopic examination. If necessary, further purification was carried out by repeating the ordinary plate culture.

Identification of yeasts. Identification was carried out according to the standard method described by LODDER in 1970 (*2*).

Results and discussion

Some examinations on the media employed

Needless to say, the selection of media and culture methods for counting and isolation is the most important matter for achieving success in this kind of experiments. The present experiments were carried out by the ordinary plate culture method mainly employing koji extract as a medium. Koji extract is a suitable medium for yeasts and molds, and is widely emloyed in Japan.

Addition of dihydrostreptomycin greatly supressed bacterial growth. The number of mold was unaffected but the number of yeasts increased by 10 to 100

Table 1. Effect of dihydrostreptomycin on the number of colonies appeared in koji extract agar.

Thirty μg per ml of dihydrostreptomycin (SM) was added to the koji extract agar.

	Yeasts			Molds		
SM addition Exp. no.	− (a)	+ (b)	ratio b/a	− (a)	+ (b)	ratio b/a
1	1.9×10^3	1.2×10^5	1.6×10^2	6.7×10^2	4.0×10^2	0.60
2	1.1×10^2	4.5×10^3	4.1×10	6.5×10^3	4.6×10^3	0.71
3	1.5×10	1.5×10^2	10	1.8×10^3	3.3×10^3	1.78
4	1.3×10^3	1.3×10^5	10^2	8.2×10^3	1.1×10^4	1.32

Table 2. Comparison of the number of colonies of bacteria which appeared in koji extract agar, soil extract agar and albumin agar.

Sample / Medium	Leaf	Leaf	Soil
Koji extract agar	1.0×10^3	0	
Albumin agar	2.6×10^4	9.0×10^2	6.1×10^5
Soil extract agar			5.9×10^5

Figures are expressed as the number of bacteria per one sheet of leaf and 1g of soil.

times under the presence of dihydrostreptomycin (Table 1). Comprarison of bacterial count with various kind of media is presented in Table 2.

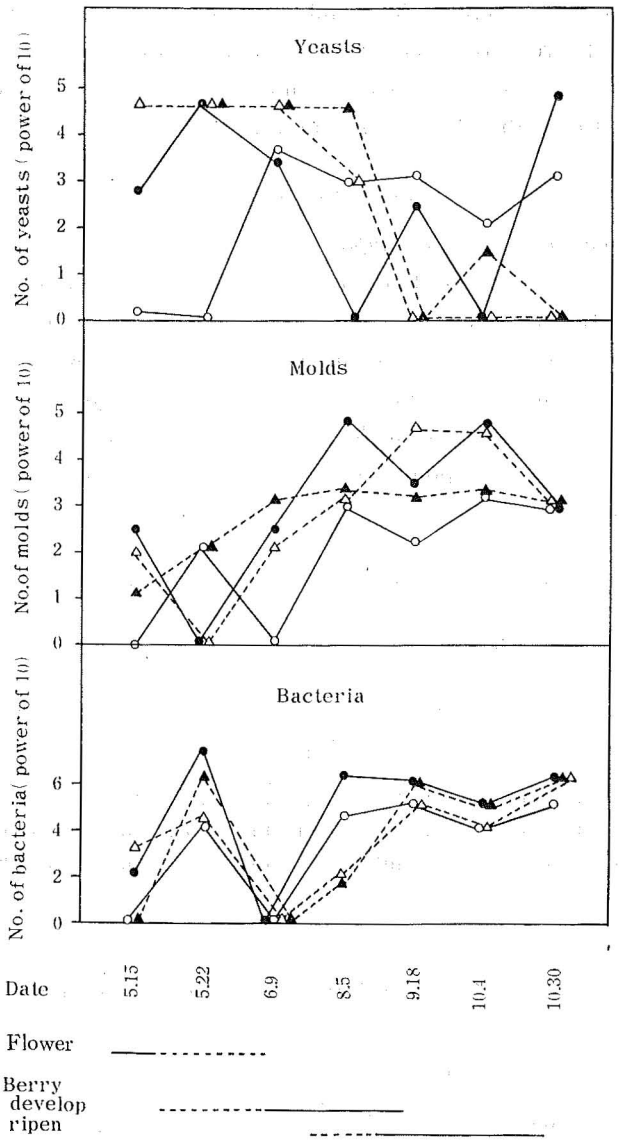

Fig. 6. Seasonal variation in the number of microorganisms from flowers and berries of goumi in 1969.

The number of microorganisms appeared in koji agar medium was counted. The number of microorganisms is expressed as the number per one piece of flower and berry. —○—, —•—, ···△···, ···▲··· : Sampling points A, B, C and D indicated in Fig. 3.

Seasonal variation in the number of microorganisms associated with the plant materials

Counting of the number of microorganisms was conducted over two years, in 1968 and 1969 from April till October, which corresponded to the flowering and fruiting time of the goumi. It was also conducted at several sampling points in the colonies of goumi indicated in Fig. 3. The winter season was excluded in this experiment.

The seasonal variations in the number of microorganisms from the flowers and berries of goumi are presented in Fig. 6.

The number of bacteria in the floweres and berries indicated nearly difinite patterns, as shown in Fig. 6. This tendency coincided with each other both in many sampling points and in the survey over a two-year period. Surprisingly, it suddenly fell to zero on May 7, 1968 and on June 9, 1969. Heavy rain-fall which will wash out the attached bacteria did not occur during several days including the sampling day. In this period, the flowers of goumi had almost fallen and tiny unripe berries could be seen. The relation between the number of bacteria and the condition of niche is not uncertain.

The humidity of the atmosphere of these dates was about 50% which is far lower than the usual humidity. A significant correlation between the number of bacteria and the humidty seemed to exist*. In some cases, the number of bacteria fell into 0 occasionally, for example, on Oct. 4, 1969 in place A and C on the leaves of goumi (Fig. 8). The humidity of this date was not so low. Accordingly, the other factors governing the bacterial growth might be present. The relation between the climatic conditions and microbial growth will be a future problem to be elucidated.

The patterns of variation in the number of mold from flowers and berries

Table 3. The number of microorganisms from attached and fallen mature berries.

	Number of microorganisms	
	Attached berries	Fallen berries
Yeasts	6.2×10	1.8×10^4
	1.9×10^3	1.0×10^6
	1.0×10^4	1.0×10^6
Molds	2.0×10^3	6.5×10^3
	1.8×10^3	6.3×10^2
Bacteria	3.3×10^3	1.5×10^4
	3.5×10^2	1.4×10^4

The number of microorganisms is expressed as the number per one piece of berry.

* Unpublished experiment.

also seemed to be definite as to the sampling points and the sampling years (Fig. 6).

On the contrary, the variation in the pattern of the number of yeasts was remarkably complicated and a distinct pattern could not be obtained as shown in Fig. 6. The pattern of variation showed great difference not only in the sampling places but in the sampling years.

This means that there might be many factors controlling the growth of yeasts. It can be seen at least, however, that the number of yeasts from berries and flowers became low during early autumn, and increased remarkably in September and October, corresponding to the period of maturation of berries (Fig. 6).

The number of yeasts was much greater in fallen berries than attached mature berries (Table 3). The fallen berries might be considered to be in an advanced state of maturity, so they might support the growth of the yeasts. Although the

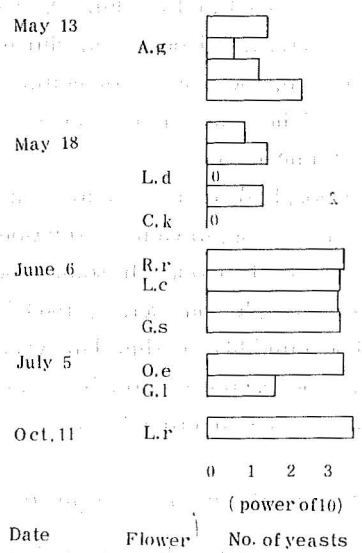

Fig. 7. The number of yeasts from various flowers in 1968.

Number of yeasts expressed as the number per one piece of flower.

The name of flowers is abbreviated as follows :

A. g : *Arabis glabra* (L.) Bernh. (Hatazao)
C. s : *Calystegia soldanella* (L.) Roem et Schult (Hamahirugao)
C. k : *Carex kobomugi* Ohwi (Kobomugi)
G. l : *Glehnia lottoralis* Fr. Schm (Hamabofu)
L. d : *Lactuca dentata* Makino (Nigana)
L. r : *Lactuca repens* Maxim. (Hamanigana)
L. c : *Lotus corniculatus* L. var. *japonicus* Regel (Miyakogusa)
O. e : *Oenothera erythrosepala* Borbas (Oomatsuyoigusa)
R. r : *Rosa rugosa* Thunb. (Hamanasu)
E. u : *Elaeagnus umbellata* Thunb. (Aki gumi), is indicated in no designation in this Figure.
A. s : *Abelia serrata* Sieb. et Zucc. var. *buchwaldii* Nakai (Kibanatsukuba-neutsugi) does not appear in this Figure.

The names in parentheses are the Japanese name.

berry juice of mature goumi contains 2 to 5 % of sugar, the berries are covered with waxy pericarp. Fallen berries might be somewhat in an injured state of their pericarp, so the growth of yeasts might become easy. Although soil contained a considerable number of yeasts (Table 5), it is conceivable that the yeasts thereof might grow on the surface of fallen berries.

Although there are many contributions on the seasonal variation in yeast from flowers and fruits, they mainly dealt with the variation in the kind of yeasts during the ripening process of fruits (*1*). Concerning the variation in the number of yeasts during ripening process of fruits, relatively few examples were reported (*3* ~ *5*). BOWEN and BEECH recorded the variation in the kind of yeasts between unripe, detached and ground-rested apples (*6*).

The number of yeasts that appeared in various kind of flowers is presented in Fig. 7. Although the kind of flowers which showed their best varied from month to month, the number of yeasts generally increased in June and July. LUND pointed out that a greater proportion of flowers was colonized by yeasts in July and August than in March and April (*4*).

The seasonal variation pattern in the number of microorganisms from leaves of goumi was nearly the same in each sampling place (Fig. 8). The number of yeasts indicated complicated patterns also in this case, although not so striking as in the case of flowers and berries. The number of bacteria in the place A and D on June 9 decreased to 0. This agreed with the case of flowers.

As to the seasonal variation in the number of microorganisms from leaves of living plants, many results appeared in the literature. KERLING followed the number of microorganisms from the fodder beet leaves in the Netherlands. The number of yeasts was dominant in May and the number of bacteria increased during July and August. With the onset of autumn the numbers of all microorganisms decreased (*7*). On the contrary, DIEM, in France, showed that the number of bacteria from barley leaves was dominant in May, and the number of yeasts and other molds incrased in July (*8*). Seasonal variation in the number of yeasts from the surface of pasture grasses in New Zealand was also reported (*9*).

The number of bacteria which could be counted in koji agar was lower than that counted in the suitable medium as indicated in Table 2. In spite of this observation, a relatively large number of bacteria could be detected on flowers, berries and leaves (Figs. 6 and 8, Table 3). The number of bacteria was usually higher in flowers and berries than that of yeasts (Fig. 6). On the contrary, they were nearly in the same order in leaves (Fig. 8). This means that the sugar utilizing or the sugar tolerant bacterial flora might be dominant in these niche.

It was pointed out that the bacteria on the leaves might prepare the essential nutrients for the growth of yeasts, so the succession of the number of bacteria and yeasts took place (*8*, *10*). Succession of the number of colonizing yeasts on

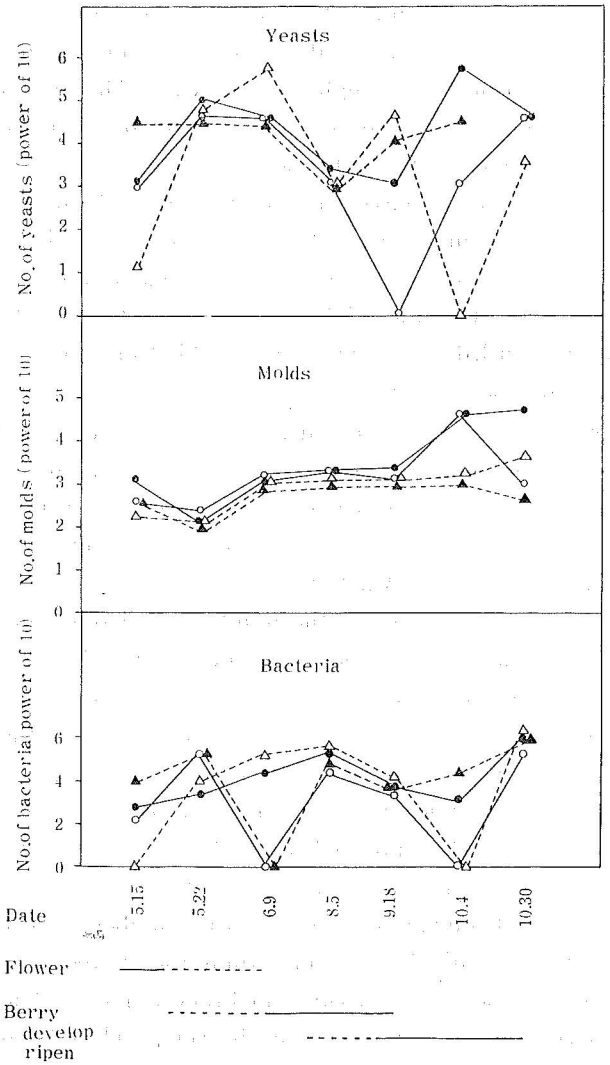

Fig. 8. Seasonal variation in the number of microorganisms from leaves of goumi in 1969.

The number of microorganisms is expressed per one sheet of leaf. Other designations are the same as Fig. 3.

leaves during aging of leaves was also indicated. The number of microorganisms, especially of yeasts, increased by aging process, for example, in strawberry leaves by KERLING (*11*), and in wheat leaves by LAST (*12*). These relations could not be observed in the present experiments.

These results including the present results do not always coincide with each other, and might be the reflection of the interaction between environmental and nutritional conditions of the phyllosphere. The climatic conditions, height of the sampling point, the airborne and soil microflora, and mediation by birds, insects

and wind *etc.* must be considered as the environmental conditions.

The number of microorganisms from soil in sandy coast

The number of microorganisms in sandy coast was also counted. Several examples are given in Table 4. The number of yeasts and molds are not always so small compared with the reported numbers of yeast (10^2~10^5 living units/g soil) (*13*~*16*) and of mold (10^3~10^5 living units/g soil) (*17*, *18*). The low number of bacteria that appeared in koji agar seems to be a natural consequence, because the high sugar content and low pH of koji medium are not suitable for the growth of bacteria.

The counting of the number of bacteria was also conducted with albumin and soil extract media which are accepted as the suitable media for the counting of soil bacteria. As shown in Table 5, the number of bacteria in sandy area was about one tenth of that in the fertilized soil, which is also too small compared

Table 4. The number of soil colonizing microorganisms in sandy coast. Koji agar was employed with or without dihydrostreptomycin (30 μg/ml).

Date	Sampling point	Yeasts	Molds	Bacteria
May 15	B	0	2.0×10^3	1.5×10^3
	C	8.6×10^2	5.4×10^3	6.0×10^3
	D	3.4×10^3	4.0×10^3	2.2×10^3
May 22	C	8.3×10^2	7.1×10^3	5.0×10^4
	D	0	9.4×10^3	9.4×10^3
Dec. 22	B	3.3×10^2	1.5×10^3	6.0×10^2
	C	3.8×10^2	8.2×10^3	1.7
	D	1.3×10^3	4.5×10^3	0
Dec. 22 (SM added)	B	6.8×10^4	2.5×10^3	0
	C	1.7×10^3	1.1×10^4	0
	D	1.3×10^5	7.4×10^2	0

Figures are expressed as the number of microorganisms per 1g of soil.

Table 5. The number of soil colonizing bacteria in sandy coast. Albumin and soil extract agar were employed.

Place	Albumin medium	Soil extract medium
Sandy coast	6.2×10^5	4.9×10^5
Erosion control forest	5.8×10^5	8.5×10^5
Fertilized farm ; the principal experimental farm of the university	5.0×10^6	6.0×10^6

Figures are expressed as the number of bacteria per 1g of soil.

with the reported bacterial number in the fertilized soil (*17*, *19*, *20*). This might be due to the fact that the sampling in the experiment shown in Table 5 was carried out in the afternoon in midsummer. The number of microorganisms in sand dune area was reported by WEBLEY *et al.* (*21*). One gram of soil from the sand dune with many grasses contained about 10^6 of bacteria and 10^5 of molds. Although the present experiments concerning the soil microorganisms were conducted at arbitrary occasions, the number of microorganisms, especially of yeasts is not so small compared with that obtained from the other kind of soils. A further systematic microbiological study concerning the soil of the sandy area is now in progress.

Identification of genera and species of the isolated yeasts

Identification of genera and species of the isolated strains was performed according to the key described by LODDER (*22*). Main characteristics of the genera which afford the ground for identification are described in the following. Although a few strains have not yet decided species and varieties, detailed physiological properties are summarized in the tables.

Hansenula H. and P. Sydow

Cells were spherical and ovoidal, reproducing by multilateral budding. Asci had the shape of the vegetative cells. From 1 to 4 ascospores were produced. Ascospores were smooth-surfaced and spherical, and usually liberated by rupture of the asci when matured. Nitrate was assimilated and fermentation was absent.

Other physiological properties are summarized in Table 6.

Saccharomyces Meyen emend. Reess

Cells were spheroidal and ellipsoidal, reproducing by multilateral budding. Asci did not rupture on reaching maturity. Ascospores were spheroidal to prolate spheroidal. Usually 1 to 4 ascospores formed per ascus. Vigorous fermentation of glucose occurred. Nitrate was not utilized. Early formation of pellicles was not observed in malt extract.

Other physiological properties are summarized in Table 6.

Schizosaccharomyces japonicus Yukawa et Maki

Cells were globose to ellipsoidal, some were cylindrical, reproducing exclusively by cross wall formation. Septate mycelium developed, in some part broke into arthrospores. Mycelial cells were growing into the agar. Free spores appeared on the agar slant. The number of spores in the ascus was usually 8 or less.

Other physiological properties are summarized in Table 6.

As to the varieties established by SLOOFF (*23*), investigations on detailed characteristics are now progressing and will be published elsewhere.

Sporobolomyces Kluyver et van Niel

Vegetative cells reproduced by simple budding. Asymmetrical ballistospores

Table 6. Physiological properties of isolates which form ascospores.

Strain number is an arbitrary one. A and K appended to the strain number indicate the year of isolation, 1968 and 1969, respectively, and a, b and c mean the new isolates resulted from repeated plate cultures.

Abbreviations of plants are the same as indicated in Fig. 7. Capital letters in head, F, L, B and Bf indicate flowers, leaves, berries and fallen berries, respectively.

Abbreviation of compounds are as follows. G : glucose, Ga : galactose, Su : sucrose, Ma : maltose, La : lactose, Ce : cellobiose, Me : melibiose, Mz : melezitose, Ra : raffinose, Sor : L-sorbose, Rh : L-rhamnose, X : D-xylose, Ar : L-arabinose, In : inulin, St : soluble starch, Il : inositol, Rib : ribitol, Er : erythritol, Et : ethylamine, NO_3 : potassium nitrate.

Strain no.	Source	No. of spore per ascus	Pseudo-mycelium	Fermentation								Assimilation								Cycloheximide* resistance	Identification of species
				G	Ga	Su	Ma	La	Ra	Me	St	NO_3	G	Ga	Su	Ma	La	X	Et		
1 K	L, E. u.	1>2	−	+	+	+	+	−	+	+	−	−	+	+	+	+	−	−	−	+	*Saccharomyces uvarum*
3 K	L, E. u.	2>1, 3, 4	−	+	+	+	+	−	+	+	+	−	+	+	+	+	−	−	+	+	*S. florentinus*
25 K	L, E. u.	2>1, 3, 4	−	+	+	+	+	−	+	−	+	−	+	+	+	+	−		+		*S. diastaticus*
22 K	B, E. u.		+	−	−	−	−	−				+	+	+	+	+	+				*Hansenula* sp.
24 K	L, E. u.		−	−	−	−	−	−				+	+	+	+	+	+				
77 K	B, E. u.	6～8	+**	+	−	+	+	−				−	+	−	+	+	−				*Schizosaccharomyces japonicus*
78 K	B, E. u.	6～8	+	+	−	+	+	−				−	+	−	+	+	−				

* Growth in the presence of 100 p. p. m. cycloheximide.

** True mycelium was observed in these 2 strains.

Table 7. Physiological properties of isolates identified as *Sporobolomyces* Kluyver et van Niel.

Strain no.	Source	True mycelium	Assimilation							Identification of species
			NO_3	G	Ga	Su	Ma	La	St	
56 K	Missing	−	+	+	+	+	+	−	+	*S. roseus*
47 bA	F, L. r	−	−	+	+	+	+	−	+	*S. pararoseus*
47 cA	F, L. r	−	−	+	+	+	+	−	+	
54 K	Missing	−	−	+	+	+	+	−	−	
40 A	F, E. u	−	−	+	+	+	+	−	−	*S. alborubescens*
47 aA	F, L. r	−	−	+	+	+	+	−	−	
74 K	L, E. u	−	−	+	+	+	+	−	−	

Abbreviations are the same as indicated in Fig. 7 and Table 6.

formed, but clump connection was not present. Salmon-pink color developed. Fermentation and starch synthesis were absent.

Other physiological properties are summarized in Table 7.

Candida Berkhout

Cells were globose, ovoid, cylindrical or elongated and reproduced by multipolar budding. Ascospores, teliospores and ballistospores did not form. Pigmentation did not occur. Fermentation was observed in many strains. True mycelium and arthrospores were absent. Pseudomycelium formed and often differentiated into pseudohyphae, blastospores and chlamydospores.

Other physiological properties are summarized in Table 8.

Cryptococcus Kützing emend. Phaff et Spencer

Cells were spheroidal or ovoidal, occasionally elongated, reproducing by multilateral budding. Inositol was assimilated. Ascospores, teliospores and ballistospores did not form. Yellow pigment formed in some. Fermentation was absent. Most strains were capsulated, capsule contained starch-like compound.

Other physiological properties are summarized in Table 9.

Rhodotorula Harrison

Cells were spheroidal, ovoidal and elongated, reproducing by multilateral budding. Ascospores and ballistospores were not formed. Red to pink pigmentation occurred. Inositol was not assimilated. Fermentation and production of starch-like compound were absent. Identification of the varieties is now under study.

Other physiological properties are summarized Table 10.

Torulopsis Berlese

Cells were globose or ovoid, some elongated, and reproduced by multipolar budding. Ballistospores, ascospores, teliospores or arthrospores did not form. Pigment was absent. Fermentation was observed in many strains. Pseudomycelium was absent. Inositol assimilation was not observed.

Other physiological propertis are summarized in Table 11.

Tabe 8. Physiological properties of isolates identified as *Candida* Berkhout

Strain no.	Source	Fermentation					Assimilation																Identification of species
		G	Ga	Su	Ma	La	NO_3	G	Ga	Su	Ma	La	Ce	Ra	Me	So	Rh	X	Ar	In	Il	Rib	Er	
60 A	B, E. u	+	−	+	−	−	+	+	+	+	+	−		+	+									*C. gelida*
2 K	L, E. u	+	+	+	−	−	+	+	+	+	+	−		+	+					+			−	*C. utilis*
27 K	B, E. u	+	+	+	−	−	+	+	+	+	+	−		+	+					+			−	
29 K	B, E. u	+	+	+	−	−	+	+	+	+	+	−		+	+					+			−	
66 A	L, E. u	+	−	−	−	−	+	+	+	+	+	−		+	+		+					+		*C. silvicola*
70 A	L, E. u	+	−	−	−	−	+	+	+	+	+	−		+	+		+					+		
58 K	Missing	−	−	−	−	−	+	+	+	+	+	+		+	+		−							*C. aquatica*
68 K	L, E. u	−	−	−	−	−	+	+	+	+	+	+		+	+		+							*C. scotti*
1 A	F, E. u	+	+	+	+	−	−	+	+	+	+	−	+	+	−	+					−		−	Haploid strain of *Pichia ohmeri*
2 aA	F, E. u	+	+	+	−	−	−	+	+	+	+	−	+	+	−	+					−		−	
2 bA	F, E. u	+	+	+	−	−	−	+	+	+	+	−	+	+	−	+					−		−	
3 aA	F, E. u	+	+	+	±	−	−	+	+	+	+	−	+	+	−	+					−		−	
3 bA	F, E. u	+	+	+	±	−	−	+	+	+	+	−	+	+	−	+					−		−	
8 aA	F, E. u	+	+	+	−	−	−	+	+	+	+	−	+	+	−	+					−		−	
8 bA	F, E. u	+	+	+	±	−	−	+	+	+	+	−	+	+	−	+					−		−	
10 aA	F, E. u	+	+	+	±	−	−	+	+	+	+	−	+	+	−	+					−		−	
10 bA	F, E. u	+	+	+	±	−	−	+	+	+	+	−	+	+	−	+					−		−	
11 aA	F, E. u	+	+	+	±	−	−	+	+	+	+	−	+	+	−	+					−		−	
11 bA	F, E. u	+	+	+	±	−	−	+	+	+	+	−	+	+	−	+					−		−	
35 A	B, E. u	+	+	+	+	−	−	+	+	+	+	−	+	+	−	+					−		−	
36 A	B, E. u	+	+	+	+	−	−	+	+	+	+	−	+	+	−	+					−		−	
39 A	F, E. u	+	+	+	+	−	−	+	+	+	+	−	+	+	−	+					−		−	
43 A	F, E. u	+	−	+	+	−	−	+	+	+	+	−	+	+	−	+					−		−	
46 A	L, E. u	+	+	+	±	−	−	+	+	+	+	−	+	+	−	+					−		−	
63 aK	F, A. s	+	−	±	−	−	−	+	+	+	+	−	+	−	±			−	+	−	−		−	*C. sake**
63 bK	F, A. s	+	−	−	−	−	−	+	+	+	+	−	+	−	±			−	+	−	−		−	

* No growth at 39°. Chlamydospores were not observed.

Abbreviations are the same as indicated in Fig. 7 and Table 6.

Table 9. Physiological properties of isolates identified as *Cryptococcus* Kützing emend. Phaff et Spencer.

Strain no.	Source	Assimilation								Starch formation	Pigment	Identification of species
		NO_3	Il	G	Ga	Su	Ma	La	Er			
61 aK	F, A. s	−	+	+	+	+	+	+		+	+*	*Cr. laurentii*
fsK	B, E. u	−	+	+	+	+	+	+		+	+	
72 K	L, E. u	−	+	+	+	+	+	+		+	+	
29 A	F, R. r	+	+	+	+	+	+	+		+	+	*Cr. albidus*
13 K	Missing	+	+	+	+	+	+	+		+	+	
32 K	Missing	+	+	+	+	+	+	+		+	+	
59 K	Missing	+	+	+	+	+	+	+		+	+	
61 bK	F, A. s	+	+	+	+	+	+	+		+	+	
17 A	F, E. u	+	+	+	+	+	+	+		+	+	
14 K	Missing	+	+	+	+	+	+	+		+	+	
30 K	Bf, E. u	+	+	+	+	+	+	+		+	+	
36 K	L, E. u	+	+	+	+	+	+	+		+	+	
47 K	L, E. u	+	+	+	+	+	+	+		+	+	
48 K	L, E. u	+	+	+	+	+	+	+		+	+	
51 K	Missing	+	+	+	+	+	+	+		+	+	
16 A	F, E. u	+	+	+	+	+	+	+		+	±	
18 A	F, E. u	+	+	+	+	+	+	+		+	±	
20 A	F, E. u	+	+	+	+	+	+	+		+	±	
27 A	F, G. l	+	+	+	+	+	+	+		+	±	
31 A	F, O. e	+	+	+	+	+	+	+		+	±	
37 A	F, E. u	+	+	+	+	+	+	+		+	±	
69 A	L. E. u	+	+	+	+	+	+	+		+	±	
71 A	L. E. u	+	+	+	+	+	+	−		+	±	
6 K	F, E. u	+	+	+	+	+	+	+		+	±	
72 A	Soil	+	+	+	+	+	+	−		+	++**	Unidentified

* Degree of pigmentation, + : slightly pigmented, ± : almost hyaline.

** Orange colored.

Abbreviations are the same as indicated in Fig. 7 and Table 6.

Yeast flora associated with the plant materials in sandy coast

Although several strains remained unable to identify species and varieties, distribution of yeast genera on the plant materials is recorded in Table 12.

Briefly summarized, a number of *Candida* yeasts was found in the flowers, berries and leaves of goumi and in the flowers of other plants. *Torulopsis* yeasts existed mainly in the flowers and berries of goumi. Yeasts belonging to *Rhodotorula*, *Cryptococcus* genera were found in all materials, and in all the period round the survey. *Sporobolomyces* yeasts were mainly obtained from leaves. Unexpectedly, ascosporogenous yeasts were isolated from leaves, although the number of isolates

Table 10. Physiological properties of isolates identified as *Rhodotorula* Harrison.

Strain no.	Source	Assimilation										EV	Identification of species
		NO_3	G	Ga	Su	Ma	La	Ra	Mz	Me	Il		
22 A	F, E. u	−	+	+	+	+	−	+	+		−		*Rh. rubra*
5 K	F, E. r	−	+	+	+	+	−	+	+		−		
55 K	Missing	−	+	+	+	+	−	+	+		−		
26 K	L, E. r	+	+	+	+	+	−	+	−		−		*Rh. graminis*
14 A	F, E. r	(+)	+	+	+	+	−	+	+	+	−		*Rh. lactosa*
38 aA	F, E. r	+	+	+	+	+	−	+	+	+	−		
51 bA	B, E. u	+	+	+	+	+	−	+	+	+	−		
64 aA	Soil	+	+	+	+	+	−	+	+	+	−		
28 K	B, E. u	(+)	+	+	+	+	−	+	+	+	−		
31 K	B, E. u	(+)	+	+	+	+	−	+	+	+	−		
57 K	Missing	+	+	+	+	+	−	+	+	+	−		
24 A	F, C. s	+	+	+	+	+	−	+	+	−	−	−	*Rh. glutinis* var.
25 A	F, C. s	+	+	+	+	+	−	+	+	−	−	−	*glutinis*
30 A	F, G. l	+	+	+	+	+	−	+	+	−	−	−	
38 bA	F, E. r	(+)	+	+	+	+	−	+	+	−	−	−	
54 A	B, E. r	(+)	+	+	+	+	−	+	+	−	−	−	
63 A	Bf, E. r	(+)	+	+	+	+	−	+	+	−	−	−	
19 A	F, E. r	+	+	+	+	+	−	+	+	−	−	+	
51 aA	B, E. r	+	+	+	+	+	−	+	+	−	−	+	
51 cA	B, E. r	+	+	+	+	+	−	+	+	−	−	+	
44 K	B, E. r	+	+	+	+	+	−	+	+	−	−	+	
12 A	F, E. r	(+)	+	+	+	+	−	+	+	−	−	+	*Rh. glutinis* var.
15 A	F, E. r	(+)	+	+	+	+	−	+	+	−	−	+	*dairenensis*
59 A	B. E. r	(+)	+	+	+	+	−	+	+	−	−	+	
64 bA	Soil	(+)	+	+	+	+	−	+	+	−	−	+	
65 aA	Soil	(+)	+	+	+	+	−	+	+	−	−	+	
65 bA	Soil	(+)	+	+	+	+	−	+	+	−	−	+	

Abbreviations are the same as indicated in Fig. 7 and Table 6. In addition, EV : external vitamins required (+) or not required (−) for growth. Nitrate was strongly assimilated : +, or weakly assimilated : (+).

was small. Beside them, many *Pullularia* (*Aureobasidium*) *pullulans*, yeast-like organism, were found in many materials.

Seasonal variation in the number of isolated yeast in relation to genera was summarized in Table 13.

As the flower colonizing yeasts, *Saccharomyces*, *Hansenula*, *Candida*, *Kloeckera*, *Rhodotorula* and *Torulopsis* were reported (*4*, *24*～*27*).

Saccharomyces, *Hansenula*, *Candida*, *Cryptococcus*, *Kloeckera*, *Rhodotorula* and *Torulopsis* were indicated to colonize on the fruits or berries, although the kind of colonizing yeasts was different according to the maturity of fruits (*1*, *4*, *28*, *29*).

Table 11. Physiological properties of isolates identified as *Torulopsis* Berlese.

Strain no.	Source	Fermentation					Assimilation										Identification of species
		G	Ga	Su	Ma	La	NO_3	G	Ga	Su	Ma	La	X	Ce	Ra	Mz	Il	
41 A	F, E. u	+	−	+	−	−	+	+	+	+	+	−		+			−	*T. ernobii*
42 A	F, E. u	+	−	+	−	−	+	+	+	+	+	−		+				
48 A	F, L. r	+	−	+	−	−	+	+	+	+	+	−		+				
21 A	F, E. u	+	−	+	−	−	−	+	+	+	+	−	+	+			−	*T. candida*
49 A	F, L. r	+	−	+	−	−	−	+	+	+	+	−	+	+			−	
50 A	B, E. u	+	−	+	−	−	−	+	+	+	+	−	+	+			−	
52 A	B, E. u	+	−	+	−	−	−	+	+	+	+	−	+	+			−	
62 A	Bf, E. u	+	−	+	−	−	−	+	+	+	+	−	+	+			−	
5 A	F, A. g	+	−	+	−	−	−	+	+	+	+	−	−		+	−	−	*T. colliculosa*
6 A	F, A. g	+	−	+	−	−	−	+	+	+	+	−	−		+	−	−	
7 A	F, A. g	+	−	+	−	−	−	+	+	+	+	−	−		+	−	−	
9 A	F, E. u	+	−	+	−	−	−	+	+	+	+	−	−		+	−	−	

Abbreviations are the same as indicated in Fig. 7 and Table 6.

Table 12. The kind and number of yeasts associated with various plant materials in sandy coast.

	Flower		Berry	Leaf		Soil	Missing	Total	Fermentable	NO_3 assimilable
	Goumi	Others	Goumi	Goumi	Others					
Ascosporogenous yeast			3(1)	4(1)				7	5	3
Candida	13	2	10	5(2)			1(1)	31	28	8
Cryptococcus	6	2	2	6	3	1	5	25	0	21
Rhodotorula	10	2	9		1	4	2	28	0	25
Torulopsis	4	4	4					12	11	4
Sporobolomyces	4			1	0		2	7	0	1
Total	37	10	28	16	4	5	10			
Fermentable	17	5	14	6	1	0	1			
NO_3 assimilable	16	3	14	11	4	5	8			

Figures indicate the number of strains isolated in 1968 and 1969. Figures in parentheses indicate the number of unfermentable yeasts.

Table 13. Seasonal variation in the number and kind of yeast strains isolated in sandy coast.

Genus	May	June	July	Aug.	Sept.	Oct.
Candida	14	2	2			3
Cryptococcus	4	5	2	2	3	5
Rhodotorula	5	5	1	1	2	9
Torulopsis	6	1				6
Sporobolomyces	1					4

Figures indicate the number of strains isolated in 1968 and 1969.

As to the leaf colonizers, *Sporobolomyces*, *Cryptococcus*, *Rhodotorula*, *Torulopsis* were reported (*7*～*9*, *12*, *30*～*32*). The present experiments agreed almost with these results, although some of these yeasts could not be detected.

Sporobolomyces yeasts discharged ballistospores violently by the drop-excretion method. They are a representative leaf colonizer and the relation to the airborne flora is discussed by many workers (*1*). On the other hand, there are few records of the presence of these yeasts on flowers and fruits except that LUND detected these yeasts on ripe barley grains (*33*). In the present experiments, these yeasts could be found not only on leaves but on flowers, although the number of isolates was small.

Cryptococcus and *Rhodotorula* were detected as the soil inhabited yeasts, but the number of isolates was too small, so they were excluded from the present discussion.

In conclusion, the kind of yeasts from plant materials in the sand dune area in question is not so different compared with that detected in plant materials in many other countries having different climate.

In the next place, the authors would like to consider on some physiological properties of the isolated yeasts. It is reasonable that *Candida*, *Torulopsis* yeasts isolated from flowers and berries which were supposed to contain much fermentable sugar, were fermentable, and these yeasts isolated from leaves were not fermentable.

On the other hand, it is interesting that unfermentable strains of *Candida* and *Torulopsis* yeasts were also found in flowers and berries. Many of these isolates had nitrate assimilating ability and this property seemed to be in reverse relation to the fermentability (Table 12). The ecological significance of these results might be a future problem to be elucidated.

In 1931. YUKAWA and MAKI isolated a new kind of *Schizosaccharomyces* yeasts from fermenting strawberry juice, and nomenclatured as *Schiz. japonicus* Yukawa et Maki (*34*). WICKERHAM and DUPRAT, in 1945, also found nearly the same yeast in home canned grape juice in Michigan, and nomenclatured as *Schiz. versatilis* Wickerham et Duprat (*35*). SLOOFF examined thoroughly the characters of both

strains and unified into the same species, but descriminated as varieties differing only in the shape of ascospores. SLOOFF gave the generic names to these yeasts as *Schiz. japonicus* var. *japonicus* Yukawa et Maki and *Schiz. japonicus* var. *versatilis* (Wick. et Duprat) Slooff (23). Since 1931, *Schiz. japonicus* were found in many districts in the world. In Japan, SAITO and OHTANI (*36*) and YONEYAMA (*37*~*40*) found this yeast in Shizuoka-ken and Hiroshima-ken, moderate climatic regions in Japan. On the contrary, KODAMA isolated this yeast from the exudate of tree in North-eastern provinces of Japan (*41*).

The present authors could also isolate this yeast from the ripe berries of goumi in North-eastern provinces of Japan. Conducting further experiments, they could isolate many strains of this yeast from exudate of broad-leaved trees*. Although KODAMA obtained merely *Schiz. japonicus* var. *japonicus*, the present authors obtained both varieties in these provinces. Detailed discussion will be made in the separate article which will be published elsewhere.

Beside the distinctive morphological features, the physiological characters of *Schizosaccharomyces* yeasts are also very distinctive from other yeasts. They require many kinds of vitamins and other growth factors for abundant growth (*42*~*44*) and can not utilize carbon sources except a few kind of carbohydrate (*45*, *46*). It is very interesting that these nutritionally exacting yeasts are growing in nature, although the materials from which the yeast in question was isolated contain abundant nutrients. It is inconceivable, however, that these nutritionally rich conditions exist throughout the year. It is also an interesting problem from the ecological view point how these yeasts survive in the nutritionally poor environment.

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摘　　要

東北日本海沿岸地方における植物付着酵母

林部正也・三戸信人・粟野省三・
倉沢諄次・高橋新紀
山形大学農学部応用微生物学研究室

東北日本海沿岸地方は，広域にわたる砂丘地帯であり，砂防のためグミその他の植物が植えられている．また，気候も内陸地方や太平洋沿岸とは異なり独特である．

このような環境条件で，如何なる種類の酵母が植物に付着し，またその数は如何なる季節的変動を示すかについて調査した．調査は1968，1969年の2年にわたり，4～10月に行なった．微生物数の季節的変動は，主としてグミの花・実・葉を対象とし，カビ・バクテリアについても同時に調査した．カビとバクテリア数の季節的変動は，ほぼ一定のパターンを示したが，酵母数の変動は極めて不規則で，一定のパターンが得られなかった．上述のような生息箇処では，酵母の生育は極めて多くの因子によって支配されていると推定した．その他の植物・土壌についても，随時微生物数を調査した

この調査期間に，上述の試料より多数の酵母を純粋分離し，これらを同定した．花・実には *Candida, Cryptococcus, Rhodotorula, Torulopsis, Sporobolomyces* が，葉には *Candida, Cryptococcus, Rhodotorula, Sporobolomyces* が見いだされた．これらの酵母は多くの研究者が世界各地で見いだした酵母の種類と大差なく，上述の環境条件でも，植物付着酵母に，とくに大きな特色はないと結論した．

Sporobolomyces 属酵母は従来葉に生息すると述べられているが，われわれは，この酵母が花にも存在することを見いだした．また少数ではあるが，*Schizosaccharomyces japonicus* のような栄養要求の著しい酵母の存在をも示した．